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The Use of Tevagrastim (Biosimilar Filgrastim XMO2) for Hematopoietic Stem Cell Mobilization in HLA Matched Sibling Donors for Allogeneic Stem Cell Transplantation to AML/MDS Patients

Ivetta Danylesko, Rina Sareli, Nira Bloom-Varda, Ronit Yerushalmi, Noga Shem-Tov, Yulia Volchek, Avichai Shimoni, Arnon Nagler. Division of Hematology and Bone Marrow Transplantation, Chaim Sheba Medical Center, Tel-Hashomer, Israel

Introduction: G-CSF Filgrastim is widely used for the mobilization of $CD34^+$ HSC. The experience with biosimilar G-CSF agents is limited. We initiated a prospective study assessing Tevagrastim (biosimilar Filgrastim XMO2) for mobilization of $CD34^+$ PB HSC in MRD for alloSCT in 24 pts with AML/MDS (NCT01542944).

Materials and methods: The study was approved by the National Regulatory Authorities and both patients and donors signed an informed consent. The donors, median age 46 years (range, 25–64), F- 14; M- 10 received Tevagrastim (10 μg/kg) BW s.c. BID for 4 days. On the morning of the 5th day they underwent conventional leukapheresis. The target yields of CD34 cell was 5×10^6 $CD34^+$ cells/kg BM of the recipient. The conditioning was myeloablative Bu/Cy (n=10), reduced toxicity Flu/Treo (n=7), Flu/Bu4 (n=3) or RIC Flu/Bu2 (n=4).

Results: Efficacy: 77-1982 $\times 10^6$ (median 749 $\times 10^6$) $CD34^+$ were collected. The number of $CD34^+$ cell per kg BW of the pts was 0.93-35.4 $\times 10^6$ (median 10.2 $\times 10^6$). Collections contained 144-709 $\times 10^8$ (median 299 $\times 10^8$) $CD3^+$ T-cells, 1.74-11.6 $\times 10^8$ (median 4.4 $\times 10^8$) per kg BW of the pts and 0.3-11.2x10^7/kg (median 2.3x10^7/kg) $CD3^-$ $CD56^+$ $CD16^+$ NK cells. The mean number of leukapheresis procedures was 1.3. Engraftment was: ANC $>0.5\times 10^9$/L and $>1\times 10^9$/L within a median of 13 days (range, 10–21) and 13.5 days (range, 10–22), respectively. PLT reached counts of $>20\times 10^9$/L and $>50\times 10^9$/L within a median of 16 (range, 12–33) and 17 (range, 12–33) days, respectively. The median days of isolation was 10 (range, 6-21). The median number of PC and PLT transfusions was 5 (range, 2-20) and 21 (range, 0-180), respectively. 20/24 (83.3%) pts showed full donor chimerism at 1 month after transplantation, respectively.

Safety: 12/24 donors reported transient arthralgias and 2 developed flu-like syndrome. The main side effects were mucositis (n-15, grade II -9, grade III-IV- 6), infections (n-20) and fluid retention (n-8). One pt suffered from VOD (Grade-I) and 5 pts developed aGVHD (grade II-III) which responded to conventional therapy. In total TRM was 1/24 at d 100. Total 5 pts died from leukemia progression during median 7 (range, 1-16) months; 2 of them died before d 100. 4 pts suffered from chronic, mild GVHD.

Conclusions: Our study with 24 AML/MDS pts, indicates that the G-CSF biosimilar XM02, Tevagrastim is safe and efficient for stem cells mobilization in MRD. The CD34 yield and post transplantation engraftment are similar to those achieved with the human recombinant G-CSF Filgrastim. We have not seen significant differences in the graft $CD34^+$, $CD3^+$ T and $CD16^+$ NK cell count, the number of leukapheresis procedures and the regeneration of WBC, neutrophils and PLTs in comparison with our historical controls. All patients promptly engrafted, and the donors developed only expected side effects. Neither graft rejection nor side effects occurred more frequently than expected from the standard G-CSF.

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CD137 Expression Identifies Leukemia Specific CTL after in Vitro Priming of Cord Blood T Cells Previously Expanded By CD3/CD28 Co-Stimulation

Jeyaraj Antony, Xiaohua Chen, Paul Szabolcs. Pediatrics, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA

Introduction: We previously reported that cord blood T-cells (CBT), obtained from <3% of cord blood graft can be expanded, and primed against (lymphoid and myeloid leukemia cell lines in the presence of IL12, IL7 and IL15 (*Cancer Research*, 2010; 70(13): 5249). Recently, we demonstrated that IL15 alone can support the priming and expansion of CTL.

Hypothesis: CD137 (4-1BB) is a member of the TNFR- family, expressed on activated T cells, which augment the survival and proliferation. Here we tested the hypothesis that de-novo CD137 expression may identify leukemia-specific CTL.

Methods: IM9-specific CTL was generated from pre-expanded CBT cells using weekly stimulations with irradiated targets and IL15. After 3 weeks, cytotoxicity was tested, re-stimulated to sort CD137 expressing cells (BD Biosciences) and were re-tested for cytotoxicity and TCRβ CDR3 spectratyping was done to identify their clonality. RT^2 $profiler^{TM}$ PCR array human Th1 & Th2 responses (SABiosciences) were employed to identify the differential gene expression.

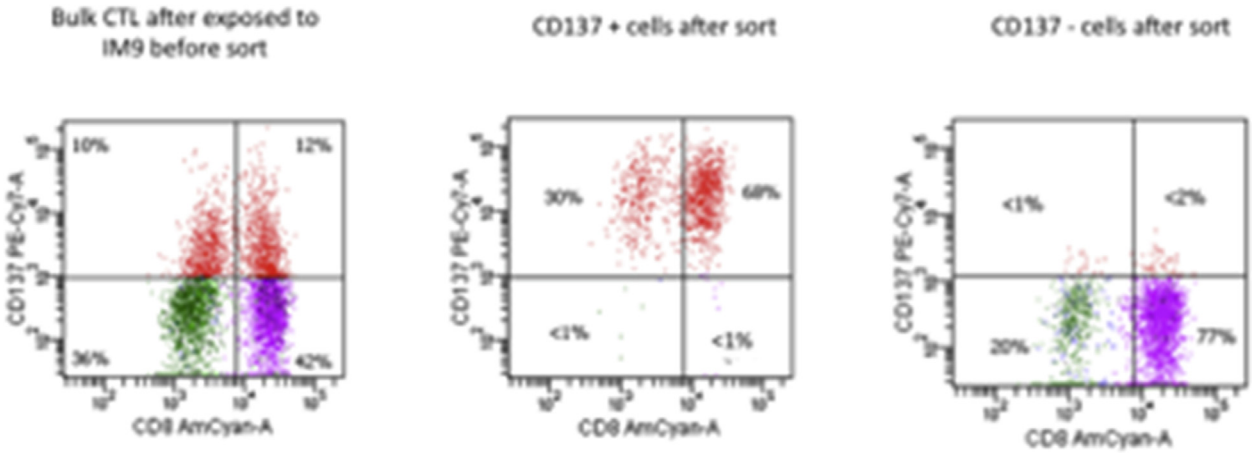

Figure 1. Representative dot plot showing the expression of CD137 on CTL before and after sort

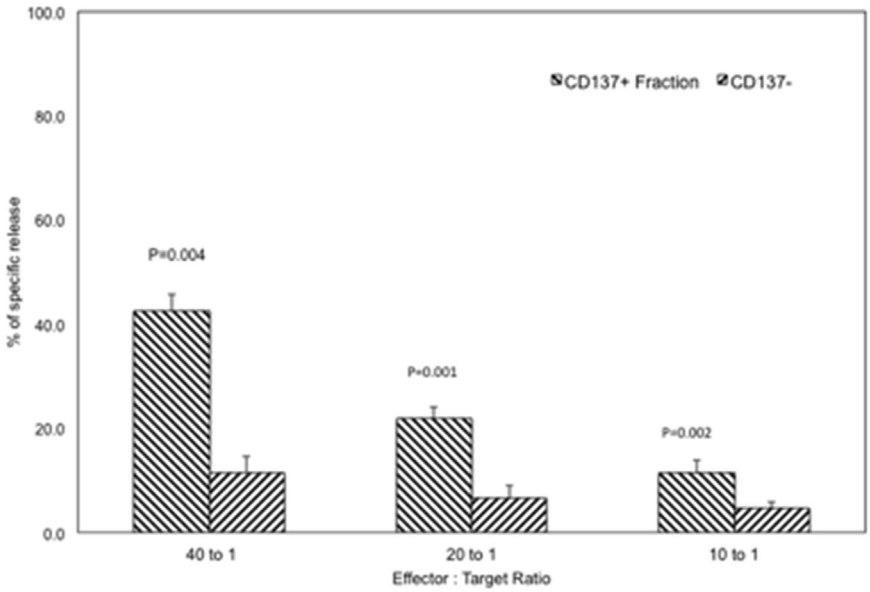

Figure 2. The cytotoxicity of CTL generated against IM9 cells using expanded CBT and IL 15 resides in CD137+ cells (mean ± SEM, n=5)

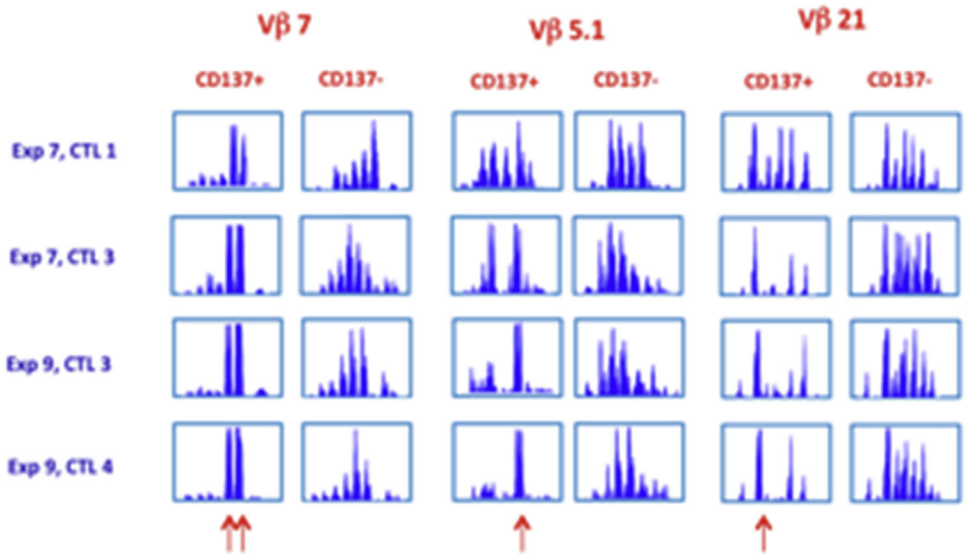

Figure-3. Oligoclonal expansion of TCR Vβ 7, Vβ 5.1 and Vβ 21 observed in isolated CD137+ cells.

Results: After 3 weeks of priming/expansion, 2-3 % of the CTL were CD137+. However, after overnight re-stimulation, CD137 expression increased to a range of 12-39% (Figure-1). The cytotoxicity of bulk cells segregated into the CD137+ cells after FACS sort (Figure-2). Amongst the 23 TCR Vβ families tested, distinct oligoclonal expansion of Vβ7, Vβ5.1 and Vβ21 were observed in the CD137+ population (Figure-3). While post-sort RT qPCR analysis demonstrated significantly increased message for CCR4, EBI3, TNFRSF9 and TNFSF4 genes, CD40LG, IL2, IL6, IL7R, SFTPD and SPP1 expressions were significantly decreased in CD137+ cells with high frequencies.

Conclusion: Cytotoxicity against leukemia cell line from expanded cord blood cells dominates in the CD137 positive subset, therefore CD137 positive selection can be used to isolate and enrich leukemia specific CTLs. Notably, CTL expanded from different sources have a predictable TCRVβ repertoire shaped by the leukemic target. These findings will have great influence on attaining clinically applicable leukemia-specific CTL protocols.

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Comparative Analysis of Cell Dose and Viability of Cord Blood Units at Cryopreservation and at Thaw/Infusion for Unrelated Stem Cell Transplantation in Adult Recipients

Spyridoula Vasileiou [1], Ioannis Baltadakis [1], Fotios Panitsas [1], Ifigenia Tzannou [2], Zoi Poulopoulou [1], Eirini Bika [1], Maria-Helena Karatza [1], Marina Papageorgiou [1], Stavros Gigantes [1], John Apostolidis [1], Nikos Harhalakis [1], Dimitris Karakasis [1]. [1] Hematology and Lymphomas-Bone Marrow Transplantation Unit, Evangelismos General Hospital, Athens, Greece; [2] Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital, The Methodist Hospital, Houston, TX